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硕士学位论文

# 好氧反硝化工艺低温生物脱氮与功能菌耐 冷机理研究

Research on Biological Nitrogen Removal by Aerobic  
Denitrification Process at Low-temperature and the  
Psychrolerant Mechanism of Functional Bacteria

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## 摘要

本研究针对传统生物脱氮工艺存在能耗大、基建和运行费用高、低温运行效果差等缺陷,采用好氧反硝化工艺处理人工模拟含氮污水,实现好氧条件下同一反应器内全程生物脱氮过程。采用逐步提高曝气时间及曝气强度的方式,以  $\text{KNO}_3$  为氮源,以普通城市污水处理厂的活性污泥为接种泥,实现了好氧反硝化菌的富集及工艺的常温启动。在此基础上,研究了温度对好氧反硝化工艺的性能影响,及低温环境下反应器运行条件的调控效果。此外,针对分离得到的异养硝化-好氧反硝化纯菌的耐冷特性,通过测定其在短期降温过程中 ATP 含量的变化情况,结合菌株在不同温度下的脱氮性能,探究好氧反硝化菌的耐冷机理。具体研究结论如下:

(1) 通过逐步提高曝气时间和强度的方式,实现了好氧反硝化菌的富集。在驯化好氧反硝化菌过程中,以  $\text{KNO}_3$  为进水主要氮源并不断提高进水碳氮负荷以刺激好氧反硝化菌的活性。系统驯化开始到结束(122 d),体系对 COD、 $\text{NO}_3^-$ -N 和 TN 的去除率从原先 40%左右分别提高到 91.39%、99.89%和 94.47%。此时,利用荧光原位杂交检测发现活性污泥中分布存在具有好氧反硝化能力的 *Pseudomonas* spp.菌。这种驯化方式得到的好氧反硝化菌能耐受较高 DO 浓度。

(2) 通过逐步降低实验室富集成熟的好氧反硝化污泥体系的温度,研究梯度温度(25°C、15°C、10°C 和 5°C)对好氧反硝化污泥碳氮去除效率的影响。当温度在 15~25°C 时,好氧反硝化污泥对  $\text{NO}_3^-$ -N ( $174.36 \pm 38.91$  mg/L) 及 COD ( $2309.72 \pm 579.36$  mg/L) 的去除率均能达到 90%以上;当温度降至 10°C、5°C 时,保持 C/N 不变的情况下,通过降低进水  $\text{NO}_3^-$ -N ( $99.87 \pm 25.25$  mg/L、 $59.41 \pm 24.55$  mg/L) 和 COD ( $1820.42 \pm 614.79$  mg/L、 $1287.66 \pm 205.93$  mg/L) 浓度,两者去除率仍分别能维持在 80%以上。根据 Van't Hoff-Arrhenius 方程得到不同温度区间内的温度系数来判断温度对反硝化过程的影响。5~15°C 下的温度系数为 1.15,好氧反硝化过程受温度影响较大;15~25°C,通过相应温度下的  $\text{NO}_3^-$ -N 的比还原速率推断可知,此时过程不受温度影响。结合 Miseq 群落结构多样性的分析数据可知,当反应器温度从 25°C 降至 10°C 时,好氧反硝化污泥中种群数目不断增加,具有好氧反硝化特性的假单胞菌属 (*Pseudomonas*) 和副球菌属 (*Paracoccus*) 的相对丰度分别从 0.0181%、0.0176%升至 0.0327%和 0.0231%,

当温度降至 5°C 时,假单胞菌和副球菌属所占的比例均大幅降低,低温对这两类菌属的生长有明显的抑制作用,但假单胞菌属的耐冷能力高于副球菌属。

(3) 温度为  $8.5 \pm 0.8^\circ\text{C}$  且进水氮源从硝酸盐氮转变为氨氮时,好氧反硝化污泥对氨氮的去除率从 100% 下降至  $37.17 \pm 11.82\%$ 。通过对降温及氮源转化阶段污泥样品与环境条件的相关性分析,发现氮源与好氧反硝化微生物群落结构呈边缘相关性 ( $0.05 < P \leq 0.1$ ),而温度、C/N 与群落结构不相关。微生物多样性与 C/N 存在边缘相关性 ( $0.05 < P \leq 0.1$ ),未观察到微生物多样性与温度的显著相关性 ( $P > 0.1$ )。

(4) 进水 COD 与  $\text{NH}_4^+\text{-N}$  的含量分别为 1191.0~1669.5 mol/L, 50.08~53.17 mol/L (C/N=23.5~30.8) 时,在 25°C、15°C、10°C 和 5°C 下, *Pseudomonas* spp.YY-7 菌对  $\text{NH}_4^+\text{-N}$  的去除速率分别为 7.42 mg/(L·h)、7.11 mg/(L·h)、2.27 mg/(L·h) 和 0.80 mg/(L·h)。通过生物荧光法检测细胞 ATP 含量,发现单个细胞产生的 ATP 量随着温度的降低反而升高。活细胞通过 ATP 含量激增改变细胞膜脂质的成分以保持膜流动性,以及增加耐受低温的功能酶含量,这是细菌适应低温的主要应激方式。

关键词: 低温生物脱氮; 好氧反硝化; 耐冷机理; 微生物群落结构

## Abstract

The traditional technology of biological nitrogen removal tends to be costly and consume large energy, as well as be severely inhibited under cold temperature. In the present study, the aerobic denitrification was applied in the simulated wastewater treatment, which aimed to achieve nitrogen removal by simultaneous nitrification and denitrification in the same reactor. Stepwise increasing of aerobic time and dissolved oxygen (DO) concentration during acclimatization, combined with  $\text{KNO}_3$  as the nitrogen source to start up an aerobic denitrification using activated sludge as inoculum. On these bases, the effect of temperature on the performance of aerobic denitrification and the performance of the reactor operated in low temperatures were also analyzed. In addition, the psychrotolerant property of the heterotrophic nitrifying-aerobic denitrifying bacterium was investigated, by determining cellular ATP concentrations during the short-term temperature downshift period. Then, the nitrogen removal performance of the bacteria under different temperature was analyzed in order to explore the mechanism of psychrotolerant bacteria. Detailed results are listed below:

(1) Aerobic bacterium were successfully enriched by stepwise increasing of aerobic time and DO concentration. In this period,  $\text{KNO}_3$  was added as the main nitrogen source in attempt to kick-start the aerobic denitrifying reaction and stimulate aerobic denitrifiers activities. At the end of enrichment process, the removal rates of COD,  $\text{NO}_3^-$ -N, TN eventually reached 91.39%, 99.89%, 94.47% respectively. Aerobic denitrifiers similar to *Pseudomonas* spp. were existed in the activated sludge via Fluorescence in Situ Hybridization (FISH) analysis. Hence the novel cultivation method applied in the present study is convenient, effective and reliable in enriching a consortium with excellent oxygen tolerance.

(2) The effect of temperature downshift (25°C, 15°C, 10°C, 5°C) on the aerobic denitrification performance was investigated, by lowering the temperature of the established aerobic denitrifying reactor gradually. When temperature ranged from 15°C to 25°C, the removal rates of  $\text{NO}_3^-$ -N and COD were all above 90%, as the influent concentration of  $\text{NO}_3^-$ -N and COD were  $174.36 \pm 38.91$  mg/L and  $2309.72 \pm 579.36$  mg/L, respectively. When the temperature was lowered from 10°C to 5°C, according to Carbon/Nitrogen (C/N) ratio, the influent concentration of  $\text{NO}_3^-$ -N gradually decreased from  $99.87 \pm 25.25$  mg/L to  $59.41 \pm 24.55$  mg/L. That of COD



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